COLCHICINE TREATMENTS TO INDUCE POLYPLOIDY IN GRAPES

By

SARUP SINGH SEKHON

Bachelor of Science
Khalsa College, Amritsar
Punjab, India
1942

Master of Science
Punjab Agricultural College
Lyallpur, Pakistan
1945

Submitted to the faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
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Thesis Approved:

Kenneth Kimmich
Thesis Adviser

Frank B. Cross

Lowell Nordin
Dean of the Graduate School

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CHAPTER I

INTRODUCTION

Polyploids, in certain instances, are more vigorous than their diploid parents probably because the genetic traits of the polyploids are an accumulation of those contributed by their diploid parents. Many good varieties of fruits, which originated as sports, have been found to be polyploids.

Since the discovery of the use of colchicine as an agent for doubling the chromosome number, it has been used with success in different fields of agriculture - floriculture, pomology, olericulture and agronomy.

The value of polyploidy in grape improvement is evident from the fact that the superior giant fruited sports, from vinifera and other bunch grapes, are tetraploids (19). The size, vigour and quality of tetraploids make them desirable for commercial and home production. So far we have depended primarily on natural conditions for polyploid grapes. Chimeras and sports have occurred in sufficient numbers but possibilities for their artificial production have not been fully explored. In the present paper results of attempts at producing polyploid grapes by the use of colchicine treatments, are reported.

CHAPTER II

REVIEW OF LITERATURE

Review of literature shows that rapid developments in the experimental production of polyploids have been made during the last twenty years or so. Of the several methods, chemical treatments have been most widely used for doubling the number of chromosomes as they are more successful and comparatively easy to make (4).

Various chemicals have been used for doubling the chromosome number. According to Blakeslee (2) "in the classic experiments of Nemec, first reported in 1904, chromosome doubling was induced with chloral hydrate". Greenleaf (12) used indole-3-acetic acid or heteroauxin with success only in the tobacco family. Eigsti (8) taking a hint from Dustin's observations that colchicine arrested mitosis for about 24 hours, treated Allium roots and found polyploid condition in many sections. Witkus and Berger (27) found that veratrine sulphate, a mixture of alkaloids, produced effects similar to colchicine. However, colchicine, since its discovery in 1937 as polyploidising agent, has been and still is most widely used for doubling the number of chromosomes.

Colchicine is an alkaloid found in varying quantities in almost all species of the genera Colchicum. Saffron meadow or fall crocus (Colchicum autmnale) is the import-

ant source of supply for the colchicine available in the market. All parts of the plant contain varying amounts of the alkaloid with the highest concentration in the corms and matured seeds which may contain 0.6%-1.23% (9).

Colchicine arrests nuclear processes at late prophase, metaphase and anaphase. Early and mid-prophase revert back to interphase. Late prophase, metaphase and anaphase are materially affected. Spindle mechanisms are partially or wholly destroyed or their development is completely inhibited. Following this and after the concentration of colchicine in the cell subsides, the cells may recover and the nuclear processes may proceed normally (9).

Various plant parts have been successfully treated with colchicine for induced polyploidy. Of the several cases on record, using different plant parts, a selected number will be reviewed here.

Seeds

Although colchicine has no visible effect on resting cells it does exert its influence on actively dividing cells. Dormant seeds have also been successfully treated for induced polyploidy. Presumably, the chemical penetrates the seeds and remains to exert its influence when the tissues of the seed resume activity after being planted.

Paul (20) obtained good results by treating seeds of chilli (Capsicum annuum) in aqueous solution of colchicine. Treatment with 0.2% concentration for 3 days and 0.4%

concentration for 2 days gave the best success of 73% and 60% respectively.

Bohn (3), in attempts to produce tetraploid tomato plants, came to the conclusion that the most satisfactory treatment, for the varieties of Lycopersicum esculentum, was soaking of seeds in 0.4% aqueous solution of colchicine for 120 hours. For L. peruvianum both 96 and 120 hour soaking of seeds gave good results.

Myers (17) induced tetraploidy in rye plants by soaking the seeds in 0.2% or 0.4% aqueous solutions of colchicine for 24 hours and 6 hours respectively.

Growing Points

Cell division is always most active at the growing points irrespective of the age of the seedling or shoot and such tissues have been treated with success by different workers.

Beasely (1) treated growing points of cotton seedlings at the time first internode began to elongate by making a slit two centimeters below the growing point and keeping it immersed in a vial containing 0.2% colchicine solution in tap water for 24 hours. Fifty five per cent of the treated plants were tetraploids and they had greater length and diameter of lint than normal diploids.

Pearson and others (21) were able to restore the fertility of F_1 hybrids of two species, Cucurbita moschata and C. maxima, through doubling the chromosome numbers by the use of

colchicine. The technique was to germinate the seeds in pots in the dark to cause hypocotyls of the seedlings to elongate. The seedlings were then exposed to light for two days to establish vigorous growth. Whole pots containing the seedlings were then inverted over trays of 0.4% aqueous solution of colchicine so that the epicotyls were immersed. Forty eight hours exposure gave the best results.

Dermen (5) potted one year old pear seedlings, headed them back to six inches and removed all but three upper most buds. The growing points of new shoots emerging out of these buds were kept moist for five days with 1.0% aqueous solution of colchicine. Later these shoots developed large flowers with large pollen grains.

Emsweller (10) induced tetraploidy in one year old seedlings of Easter lily by exposing their meristems and then dipping them in colchicine solutions of 0.2% to 1.0% for two hours.

Detection and Determination of Polyploidy

Review of literature shows that most workers have used one or more of the following criteria for the detection and determination of polyploidy:

i. Morphological changes
ii. Increase in size of stomates
iii. Increase in size of pollen grains
iv. Chromosome counts

Morphological changes

Visible changes of size and shape of plant are the major

morphological characteristics of induced polyploidy. Some parts may show increase and others decrease. Tetraploid grapes have larger berries but shorter internodes than their normal diploid parents (19). Tetraploid cotton has greater lint length but shorter internodes than diploids(1). Hence any visible morphological abnormality or abnormalities in the treated plants as compared to diploid parents may be suspected to be due to induced polyploidy.

Size of Stomates

Increase in size of stomates has been most widely used as a criterion of polyploid nature. In cotton, size of stomates was 14% greater than those of normal plants. Tetraploids of Lilium (10), grapes (7), sugarbeets (22) and tobacco (26) had greater stomatal lengths as compared to untreated diploids.

Morphological abnormalities may fail to provide indication of induced polyploidy but increase in length of stomates furnishes definite measurable indications. Dermen (6) could not detect any morphological change in treated peach seedlings from outward appearance but determined polyploid sectors through stomatal measurements.

Size of Pollen Grains

Size of pollen grains is another and better criterion extensively used to detect polyploidy. Pollen grains in tetraploid tobacco plants were 30-40% larger than normal

diploids (26). In tetraploid pears and larkspur the grains were twice as big as in diploids (13). Tetraploid peaches had large and square shaped pollen grains while in diploids they were small and triangular (6).

In wheat crosses where polyploidy could not be detected either by morphological changes or even by the measurement of stomates, increased size of pollen grains up to 45% helped to distinguish them (25). Hence detection of induced polyploidy can more accurately be made through measurement of the size of pollen grains and it should, wherever feasible, follow the measurement of stomatal lengths.

Chromosome Counts

Polyploidy is most conclusively verified by chromosome counts. Though a sure method, its reliability depends on the region from which the sample has been taken for chromosome counts. In potatoes Johnstone (16) noted tetraploid roots but diploid tops. Einsett and Pratt (11) found that in grapes different layers of cells were diploid or tetraploid. In some plants upper layers (epidermis) were diploid while inner layers were tetraploid. In some plants the situation was reverse.

In colchicine treated blackberry seedlings Hull (14) observed shoots and roots which had either just internal tissues or only epidermal cells affected while in others various combinations of tetraploid and diploid cells existed.

CHAPTER III

METHOD AND PROCEDURE

Study was conducted in the Horticultural Department of the Oklahoma Agricultural and Mechanical College, Stillwater from December 1953 to February 1956, to determine the possibility of producing tetraploid grapes by the use of colchicine treatments.

Three varieties used in the study were:

Bailey : Strong vigorous vines with large compact clusters; berries with thin but tough skin; fair quality.

Delaware: Moderately vigorous vines with small to medium compact clusters; small light red colored berries with thin tenacious skin; good quality.

Seneca : Vigorous grower with medium size moderately compact clusters; medium sized yellowish green berries with thin tender skin; good quality.

Procedure

Of the several techniques of colchicine application, three methods used in the present study were bud paint, capsule and shoot dip treatments.

One year old wood cuttings of Bailey and Delaware were made on 9th December, 1953. They were packed in moist peat and placed in cold storage at temperature of 34°F on December

12th, 1953. On January 28th, 1954 they were removed from the storage and planted in sandy soil in rows on a bench in the greenhouse. Twelve cuttings were placed in each row. The temperature was maintained at 60°F at night and 70°F during day time.

Bud Paint Treatment

After about twelve days when buds showed signs of swelling they were painted with colchicine-lanolin paste of different concentrations. Each row was treated with a separate concentration. The proportions of lanolin and colchicine for different concentrations were as follows:

Concentration	Colchicine	Lanolin
0.2%	0.04 grams	19.96 grams
0.4%	0.08 "	19.92 "
0.6%	0.12 "	19.88 "
0.8%	0.16 "	19.84 "
1.0%	0.20 "	19.80 "
1.2%	0.24 "	19.76 "

Capsule Treatment

Buds were allowed to grow shoots 1-2 inches long. The growing points of shoots were exposed as far as possible by cutting off folded leaves. Capsules were made of Tygon plastic tubing pieces about 3/4 inch long, sealed at one end. The capsules contained cotton plugs which were saturated with aqueous colchicine solution and carefully slipped over the growing points to remain for 48 hours. However, the capsules were removed twice a day to resaturate the cotton plugs with

respective colchicine solutions. The colchicine concentrations used were 0.3%, 0.4% and 0.5% in distilled water.

Shoot Dip Treatment

For this treatment one year old plants of Seneca variety were transplanted into individual pots. Two shoots were retained and others removed. When the shoots were 5-6 inches long, the growing points were carefully exposed by cutting off folded leaves and then were dipped in vials containing colchicine solutions of 0.3%, 0.4% and 0.5% for 12, 24 and 36 hour time intervals. Aqueous solutions of colchicine of desired strengths were made and 2-4 drops of 10% Santomerse were added to reduce surface tension of the film.

Determination

From the various treatments certain individual plants evidencing morphological changes, were selected and retained. These plants were removed from the greenhouse bench, potted and identified as numbered selections beginning with Bailey variety followed by Delaware and Seneca.

To determine the effectivness of the treatments, lengths of stomates were measured as there is close correlation between the increase in chromosome number and length of stomata. Various methods of preparing leaves for stomatal observations such as boiling in absolute alcohol to remove cholorophyll or boiling in potassium hydroxide and peeling off the epidermis, failed to yield results with grape leaves of the

varieties used in this study due to the presence of heavy pubescence on their under sides. The method of obtaining impressions of leaf cells on collodion films, adapted from Sax (24) and described below worked well with grapes.

Pubescence was rubbed off by means of art gum from the area where the sample was to be taken. Collodion was painted in a thin film on this area, care being taken that the film was uniformly thick. When the edges of the film began separating it was peeled from the leaf by means of a foreceps and cemented on a clean slide. Cover slip was immediately put on and sealed by means of jewelry cement.

Uniform leaf samples were taken from the fifth node below the growing point both in case of normal and treated plants. Collodion films from uniform spots ($\frac{1}{2}$ cms. from the midrib and $\frac{1}{2}$ cm. from the sinus) were taken and slides prepared. The lengths of stomates were measured by means of a calibrated microscope. Data obtained from normal plants were subjected to statistical analysis to obtain "tolerance limits" which could be used as a yardstick to compare readings of treated plants with normals. Camera lucida drawings of sections of leaves of each variety, Bailey, Delaware and Seneca, were made to compare with those from treated leaves of the same varieties.

For detection of any increase in chromosome numbers in treated plants fresh cuttings from treated plants, having markedly different appearance, were taken and rooted in vermiculite under constant mist spray.

Roots were collected at 10.00 A. M. and fixed in Craf III for 24 hours and cross sections cut 10 microns in thickness. The sections were stained in crystal voilet as described by Sass (23).

CHAPTER IV

EXPERIMENTAL RESULTS

Bud Paint Treatment

Dormant buds of grapes are encased in scale leaves and swelling of buds is the earliest visible indication of cell activity having been resumed by the dormant buds. Accordingly buds were painted with lanolin-colchicine paste following evidence of swelling. Ultimate growth from buds, treated at this time, gave rise to shhots which apparently were not affected. It was, therefore, concluded that penetration of colchicine through scale leaves is materially retarded. Hence the growth should proceed to the point of breaking or splitting of bud scales before the treatments are applied. This would allow the colchicine to reach the growing point area.

The first leaf to expand following colchicine application, that is the outermost of those surrounding the apical dome, was invariably more distorted than those developing later on. Crumpled portions of the affected leaves were darker green than were the smoother portions of the same leaf. Each succeeding leaf showed less and less distortion until the leaves appeared to be normal. The rate of shoot elongation decreased as compared to the rate of growth of a normal shoot. The internodal length was less than that in the normal

plants (Fig. 1).

The colchicine concentration, the number of plants treated and the number affected from the bud treatments are shown in **Table I.** The lowest concentration of colchicine, 0.2%, did not affect the variety Bailey while two of the six Delaware plants treated were affected. Both were affected by the 0.4% colchicine treatments.

TABLE I

BUD PAINT TREATMENTS: CONCENTRATIONS OF COLCHICINE, VARIETIES, NUMBER TREATED AND THE NUMBER OF BUDS SELECTED.

Variety	Bailey		Delaware	
Concentration of colchicine in lanolin	Number treated	Number selected	Number treated	Number selected
0.2%	5	-	6	2
0.4%	9	4	8	3
0.6%	12	3	12	10
0.8%	10	5	10	6
1.0%	10	5	10	6
1.2%	9	2	10	6

Apices of young shoots arising from buds treated with 1.2% colchicine concentration were killed in the case of the Bailey variety but they survived in Delaware. This high concentration was more detrimental to Bailey.

Twenty measurements of stomatal lengths were made from collodion film impressions of leaves of each selection. Mean stomatal length for each selction was determined and compared with the data of untreated plants. The data on the length of stomates of five normal plants of each Bailey, Delaware and Seneca varieties were subjected to statistical analysis

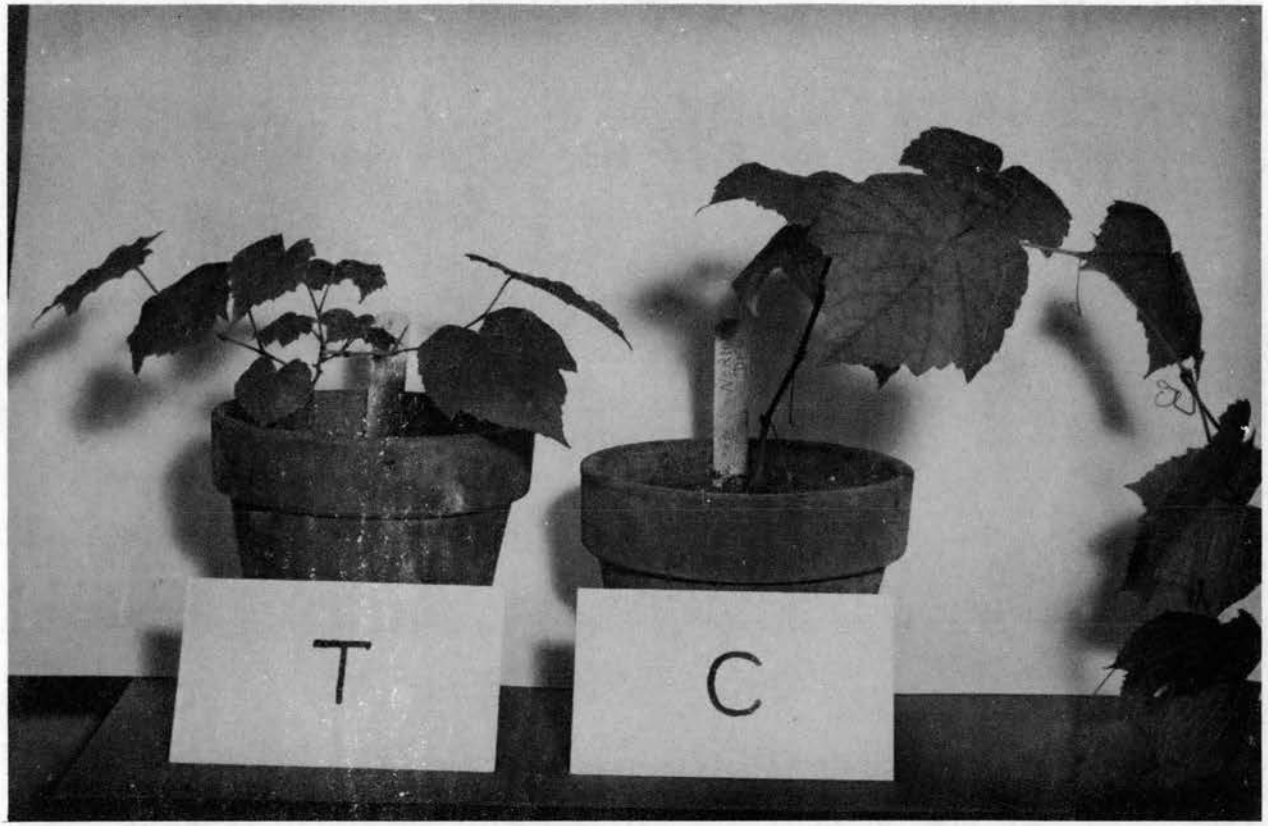


Fig. I Immediate effect of treatment (0.3% Capsule for 48 hours) on the appearance of Delaware plant.
T = treated plant
C - normal plant

and tolerance limits were calculated at the 90% level. The range or the maximum limits of variation, thus, assures that if an observed value for any selection is greater than this limit, the difference is due to the treatment. The limits as determined in different varieties are:

Variety	Minimum	Maximum
Seneca	22.30 microns	28.72 microns
Delaware	21.56 "	26.94 "
Bailey	20.83 "	25.83 "

Applying this information to other stomatal length measurements under different treatments, the plants, identified with an asterisk, were found to have readings higher than the maximum tolerance limits. None of the treated or affected plants exceeded the lower limit. Camera lucida drawings of the leaf tissues from treated and untreated grape plants show differences in size of stomates. Drawings of the representative material of treated and untreated Bailey, Delaware and Seneca plant leaf sections are presented in Fig. 2. The data on stomatal length are tabulated in Table II.

Capsule Treatment

Capsules, containing cotton plugs saturated with colchicine solutions, were slipped over carefully exposed growing points of shoots. The treatment was continued for forty eight hours. The number of growing points of shoots treated and the ones selected as having been affected by the treatments, were recorded in table III.

TABLE II

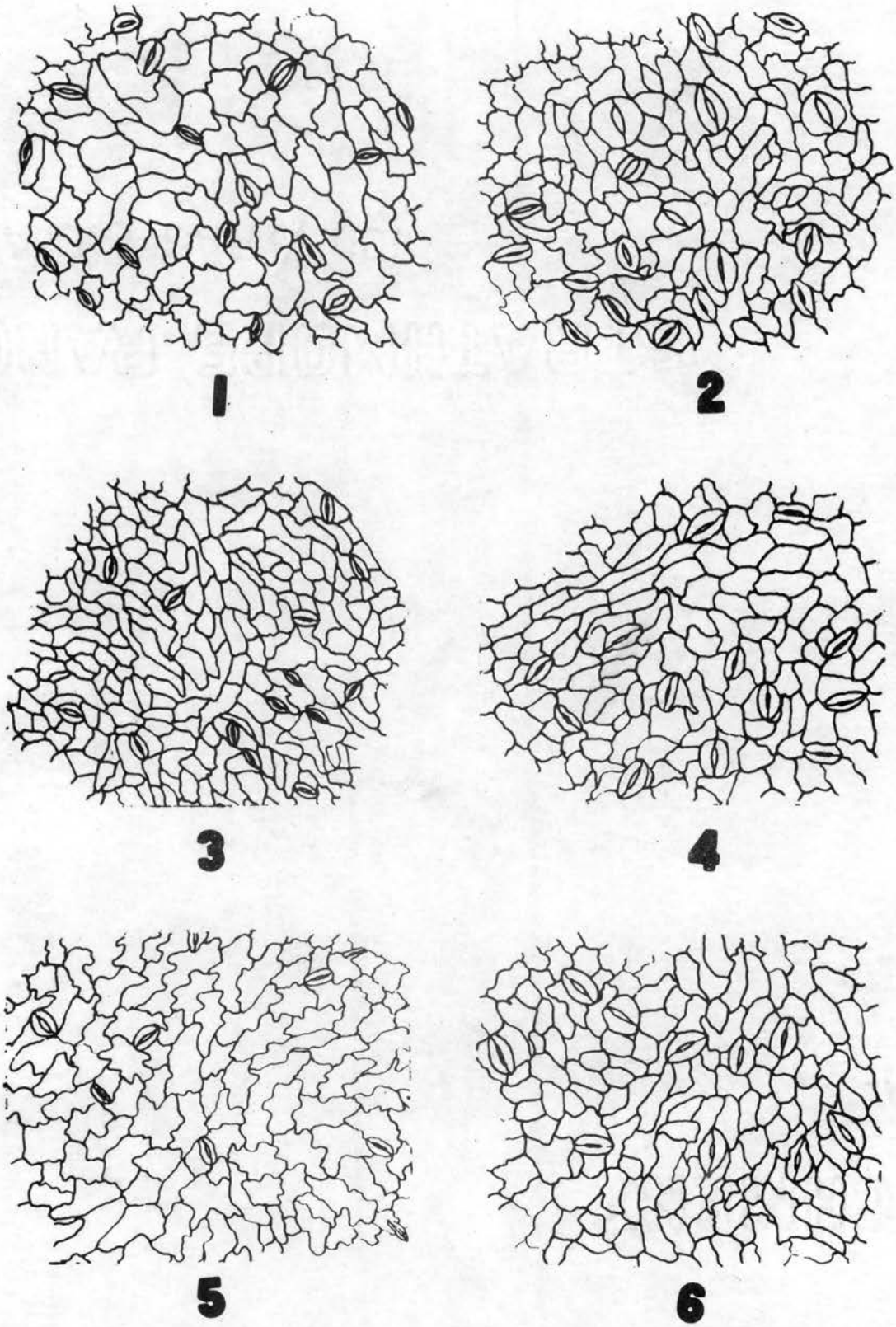
STOMATAL LENGTH MEASUREMENTS IN BAILEY AND DELAWARE GRAPE LEAVES FOLLOWING DIFFERENT TREATMENTS OF BUD PAINT

Variety	Bailey		Delaware		
Treatment	Plant number	Stomatal length in microns	Treatment	Plant number	Stomatal length in microns
0.2% B.Paint	No selection		0.2% B.Paint	26	23.31
				27	21.78
0.4% B.Paint	1	21.79	0.4% B.Paint	28	22.25
	2	21.69		29	26.28
	3	22.10		30	24.93
	4	23.10			
0.6% B.Paint	5	25.20	0.6% B.Paint	31	26.94
	6	25.60		32	27.63
	7	27.18 #		33	27.20
				34	28.53 *
0.8% B.Paint	8	25.29		35	25.38
	9	28.26 #		36	28.35 *
	10	23.04		37	26.10
	11	25.38		38	23.76
	12	24.27		39	23.40
				40	21.60
1.0% B.Paint	13	23.85	0.8% B.Paint	41	30.33 *
	14	24.39		42	28.17 *
	15	25.20		43	26.82
	16	22.95		44	22.63
				45	27.45 *
1.2% B.Paint	17	27.72 #		46	25.29
	18	23.84		47	24.30
			1.0% B.Paint	48	27.45 *
				49	28.44 *
				50	29.16 *
				51	29.70 *
				52	28.44 *
				53	24.03
			1.2% B.Paint	54	28.17 *
				55	21.69
				56	24.75
				57	23.40
				58	23.58
				59	24.66

\# - Exceed tolerance limits for Bailey

* - " " " " Delaware

Fig. 2: Camera lucida drawings of representative leaf tissues of treated and untreated Seneca, Bailey and Delaware varieties of grapes (x 450).



1. Normal Seneca
2. Treated Seneca
3. " Bailey
4. " Bailey
5. " Delaware
6. " Delaware

TABLE III

CAPSULE TREATMENT: CONCENTRATIONS OF AQUEOUS SOLUTIONS OF COLCHICINE, VARIETIES, NUMBER OF GROWING POINTS TREATED AND SELECTED

Variety	Bailey		Delaware	
Concentration of colchicine in water	Number treated	Number selected	Number treated	Number selected
0.3% for 48 hrs.	9	3	7	5
0.4% " " "	5	-	9	3
0.5% " " "	4	-	7	3

Treatments with 0.4% and 0.5% colchicine solution for 48 hours were found to be extremely injurious to the growing points. Some of the growing points treated with these concentrations failed to make further growth and subsequently died. In Delaware only 3 survived out of seven treated with 0.5% colchicine solution while in Bailey none survived the 0.4% and 0.5% treatments. All plants, which recovered, were saved for further examination. Treated plants had shorter internodes, smaller leaves and characteristic type of branching assuming a bushy pattern (Fig. 1). The leaves though thicker than normal did not develop the roughened or crumpled condition as was the case of leaves for bud paint treatment.

Collodion films from leaves of treated plants were prepared and the impressions of their stomatal lengths measured. The data are tabulated in Table IV on the following page.

TABLE IV

STOMATAL LENGTH MEASUREMENTS IN LEAVES OF BAILEY AND DELAWARE GRAPES IN MICRONS FOR DIFFERENT CAPSULE TREATMENTS

BAILEY			DELAWARE		
Treatment	Plant number	Stomatal length in microns	Treatment	Plant number	Stomatal length in microns
0.3% Capsule	20	22.53	0.3% Capsule	60	24.03
	21	25.92 #		61	24.66
	22	27.90 #		62	27.58 *
				63	24.39
				64	29.34 *
			0.4% Capsule	65	27.35 *
				66	25.57
				67	26.19
			0.5% Capsule	68	25.74
				69	28.71 *
				70	24.45

- exceeded tolerance limit 25.83 for Bailey
* - " " " 26.94 " Delaware

Shoot Dip Treatment

One year old plants of Seneca were used. The growing points of shoots were exposed, as far as possible, by cutting off folded leaves. Prepared shoots were submerged in vials containing different concentrations of colchicine for periods of 12, 24 and 36 hours, as indicated in Table V. The shoot apex receiving 12 hour treatment in 0.4% colchicine solution slipped out of the vial. Since this treatment was interrupted this shoot was not considered further. Stomatal length measurements were recorded by preparing collodion film strips of treated leaves and the data are presented in

Table V. The 0.3%, 0.4% and 0.5% colchicine concentrations were not highly toxic to the growing points of shoots and all shoots survived.

TABLE V

SHOOT DIP TREATMENT: STOMATAL LENGTH MEASUREMENTS IN LEAVES OF SENECA GRAPE IN MICRONS UNDER DIFFERENT SHOOT DIP TREATMENTS

Concentration of colchicine in water	Time interval	Number treated	Stomatal length in microns
0.3%	12 Hours	1	27.00
	24 "	1	26.28
	36 "	2 A	36.90 *
		B	29.43 *
0.4%	12 "	Treatment	interrupted
	24 "	1	29.97 *
	36 "	2 A	36.62 *
		B	27.54
0.5%	12 "	1	23.54
	24 "	1	28.34
	36 "	2 A	34.47 *
		B	25.02

* Values higher than the tolerance limits for the Seneca untreated. The range is 22.30-28.72 microns

As the treatment time was increased more cells were affected and as a result there was an increase in average stomatal length. Weaker concentrations for longer periods had similar effects.

Differences in Stomatal Lengths as Affected by different Treatments

Table VI shows a comparision of stomatal lengths from treated plants (above tolerance limits) with untreated. Of this group, the lowest percent increase was 11.2 . The average percentage stomatal length increase was

found to range from 16-20%. This percent increase in length of stomates is fairly close to what was found with tetraploid cotton. Since the increase in stomatal length is directly associated with increase in chromosome number, it could be assumed that such tissues are polyploids.

TABLE VI

PERCENT INCREASE IN AVERAGE STOMATAL LENGTH OF TREATED PLANTS *

Variety	Treatment	Ave. Stomatal length of 5 normal plants	Ave. Stomatal length of individual treated plants	Percent increase
Bailey	0.6% B.Paint	23.3 microns	27.18 microns	16.6
	0.8% "		28.26 "	22.7
	1.2% "		27.72 "	19.8
	0.3% Capsule		25.92 "	11.2
			27.90 "	20.6
Delaware	0.6% B.Paint	24.2 "	27.63 "	14.1
			27.20 "	12.3
			28.53 "	17.8
			28.35 "	17.1
	0.8% "		30.33 "	25.3
			28.17 "	16.4
			27.45 "	13.4
	1.0% "		27.45 "	13.4
			28.44 "	17.5
			29.16 "	20.5
			29.70 "	22.7
			28.44 "	17.5
	1.2% "		28.17 "	16.4
	0.3% Capsule		27.58 "	13.9
			29.34 "	21.2
	0.4% "		27.35 "	13.0
	0.5% "		28.71 "	18.6
Seneca	Shoot Dip 0.3% 36 hrs.	25.5 "	36.90 "	44.7
			29.43 "	15.4
	0.4% 24 "		29.97 "	17.4
	36 "		36.62 "	43.6
	0.5% 36 "		34.47 "	35.1

* Plants exceeding the highest tolerance limit of normal checks.

Chromosome Counts

As chromosome counts are the final criteria of any induced polyploidy, attempts were made to count them. The portions of shoots beyond the point of treatment, were cut and rooted under constant mist spray. Roots, formed in about ten days, were removed, killed and fixed in Craf III. Carnoy's fluid, which was originally used, caused shrinkage of the tissues and hence was discontinued.

Roots killed and fixed about 10.00 A. M. showed cells, which were dividing. It was not possible to cut sections thinner than 10 microns since they usually broke when the ribbon was expanded in preparation for fixing it to the slide.

Though it was possible to view the chromosomes in many cells, they could not be counted, as the chromosomes in grapes are small.

CHAPTER V

DISCUSSION

In several species of plants doubling of chromosome number has caused increase in size of flowers and fruits. Seedlessness of triploids, as a result of crossing between diploids and tetraploids, is also an admitted fact of great importance. Both these desirable characters-large fruit size and seedlessness, may be combined in grapes when suitable techniques, for producing tetraploids of desirable varieties, are worked out. Literature is replete with instances where tetraploids of several species of plants have been produced by the use of colchicine.

Variable colchicine treatments were given to grapes in the present study. The effectiveness of treatment appeared to depend upon the concentration of colchicine reaching a growing point, the stage of development when colchicine reaches the cell and the time interval it remains in effective concentration in the cell.

Production and utilization of polyploids involves three steps viz., application of treatments, detection, and propagation of the affected parts. The treatments, applied in the light of the experience of other workers, have been described previously. For detection of any polyploidially induced abnormality, length of stomates has been relied upon, as an

indication of change. Stomatal length has been shown to be closely associated with chromosome number. Stomates in tetraploid grapes (7), peaches (6), lilium (10), sugar beets (22), potatoes (16) and tobacco are greater than those of normal diploids. Stomata in tetraploid cotton were 14% larger than those in diploids (1) and in apples the difference between stomatal lengths of triploids and diploids was 10% (18). Measurement of stomatal lengths provides research workers an opportunity to quickly screen numerous plants with high accuracy when other methods, such as root sections and chromosome counts, are not practicable. As the length of stomata varies with the conditions of growth, cultural conditions of plants grown for comparison must be kept as uniform as possible.

Prior to the discussion of specific data, it appears desirable to consider some immediate effects of treatments with colchicine. The growth was checked. The shoots from treated plants had shorter internodes and branched, resulting in a characteristic bushy appearance while untreated plants had little tendency towards branching and the main shoot elongated rapidly. These characters conform to the description of tetraploid grapes as reported by Olmo (19). The U-shaped leaf base character of tetraploid muscadines, as observed by Dermen (7) was not consistent or in some cases evident in the varieties of bunch grapes used in these experiments. The individual treatments may be considered separately as in the following pages:

Bud Paint Treatment

The growth of shoots is from a number of distinct cell layers at the very summit of the shoot apex. When colchicine is applied to vegetative buds, which will grow into shoots, polyploidy is induced in certain layers independently of others, so that various combinations of polyploid and normal layers are to be expected within the same meristem.

As none of the plants receiving 0.2% and 0.4% lanolin-colchicine bud paint treatment, developed leaves with stomatal lengths greater than the tolerance limits, it appears that these concentrations are not high enough to morphologically affect the apical meristem. The very young leaf and leaf primordia provide considerable protection against penetration of colchicine to the growing point. Hull (14) describes overlapping of young leaves and their heavy pubescence, which entrap air in the area adjacent to the dome, as being responsible for limited colchicine penetration.

In the Bailey variety 0.6% and 0.8% bud paint treatment gave rise to shoots which had leaves with increased stomatal lengths and epidermal tissues affected by these treatments. The fact that very few plants were affected is probably due to poor penetration or incomplete contact of colchicine with the plant tissues. On the other hand, buds of the Delaware variety painted with the same 0.6% and 0.8% colchicine concentration produced greater number of plants which appeared to be affected. This was explained, following

careful observation, by the fact that leaves of Bailey were thicker and had a heavier coating of hairs than the Delaware variety.

The fact that a greater number of Delaware plants had stomatal lengths exceeding the higher limit of tolerance than in the Bailey plants, receiving the same treatments, indicates that varieties respond differently to the same treatment and that a treatment suitable for one variety may not hold for another.

Capsule Treatment

As colchicine is in aqueous solution it is better able to reach the tissues to be treated than was possible by the bud paint method. In the latter instance the heavy pubescence formed a mat between the layer of colchicine-lanolin paste and the plant tissue which was to have been treated. This is the primary explanation of why a low concentration of colchicine in aqueous solution is quite effective in modifying subsequent growth, measured as stomatal lengths. Concentrations of 0.3% and 0.4% colchicine for 48 hours were effective. Concentrations higher than this were severely injurious to the growing points of both Bailey and Delaware varieties under treatment.

Shoot Dip Treatment

The shoot dip treatment, like the capsule treatment, affords complete contact of the chemical with the plant

tissues and concentrations of 0.3% and 0.4% for 24 hours and 36 hours were effective. Careful scrutiny of the data will reveal that weaker concentrations for longer periods and higher concentrations for shorter periods had the same effect. Longer exposure periods or higher concentrations caused a greater number of cells to be affected by colchicine.

Conclusion

It can, therefore, be concluded that bud paint treatment in concentrations of 0.6% - 1.0% in grape varieties with less pubescence and thinner leaves than the Bailey, will produce significant effects, among which are increased stomatal lengths. Lesser colchicine concentrations of 0.3% and 0.4% in aqueous solution are equally effective in causing an increase in stomatal lengths.

Since increased stomatal length is closely associated with increase in chromosome number, the percent difference between stomatal lengths of treated and normal plants is usually in the range of 16-20%. As this difference is fairly close to what is expected at the tetraploid level in cotton (14%), we can thus safely assume such tissues to be polyploid.

Caution, however, is advised in using stomatal length as the final criteria of induced polyploidy because stomata may vary considerably under various conditions of growth. Though conditions of growth, in the present experiment, were kept as uniform as possible and the data on stomatal length is comparable, still polyploids will have to be confirmed by chromosome counts.

CHAPTER VI

SUMMARY AND CONCLUSIONS

In order to explore the possibilities of producing tetraploid grapes from desired varieties, three different methods of colchicine application - bud paint, capsule and shoot dip treatments, were tried. Three varieties of bunch grapes used in the study were Bailey, Delaware and Seneca.

For treatment of buds, colchicine concentrations in lanolin varied from 0.2% to 1.2%. For treatment of growing points, aqueous solutions of 0.3%, 0.4% and 0.5% colchicine were used. Growing points were treated by two methods. First method consisted in keeping the exposed growing points immersed in 0.3%, 0.4% and 0.5% aqueous solution of colchicine for 12, 24 and 36 hours. The second method employed capsules containing cotton plugs, saturated with colchicine solutions of 0.3%, 0.4% and 0.5%, which were slipped over carefully exposed growing points. Treatments were continued for 48 hours.

For detection of induced polyploidy, visible morphological differences such as thicker, smaller and more intense green leaves and shorter internodes were used. These were regarded as preliminary selections and plants were properly identified for further observations.

The effect of colchicine on stomatal length was studied. Impressions of leaves of comparable age and similar location

were made for more detailed study of stomatal lengths. The selected spots were cleaned of pubescence and collodion films applied in order to make the impressions and from them stomatal length measurements. Tolerance limits were determined for normal plants of the three varieties under study. Calculations to determine the probabilities at the 90% level of treated and untreated plants were made. Plants, with leaves having stomatal length measurements beyond the maximum tolerance limit for normal plant, were considered to have been affected by colchicine application. The technique of preparing collodion films has been described in detail.

Application of bud paint was found to be most effective when applied at the time cracks and slits appear in the scale leaves as a result of the developing apical dome. Buds, treated at the first signs of swelling, produced normal shoots. Bud paints of 0.6%-1.0% colchicine concentration resulted in plant growth, the leaves of which produced stomata of significantly greater length than those of untreated plants. Response to bud paint treatment varies with the variety. Delaware variety was affected more than Bailey from identical treatments.

Capsule treatments, with 0.3% or 0.4% for 48 hours time interval, have been quite effective in modifying growth. Higher concentrations than these were injurious.

Dipping the exposed growing points of shoots in 0.3% or 0.4% aqueous solution of colchicine for 24 and 36 hour treatment have proved equally effective.

The stomatal lengths of colchicine treated plants were 16-20% larger than those of normal untreated plants. This difference is fairly close to what is found in tetraploid cotton (14%).

Suggestions For Future Study

1. Plants should be grown in individual containers instead of benches. This would enable better handling of individual plants and facilitate treatments. Both accuracy and efficiency of operation would be improved.
2. Temperature records and the time of treatment should be recorded to establish the time of active cell division. This is particularly important since the precise stage of cell division, at the time of colchicine application, accounts for the success or failure of the treatment. Cells, in early and mid-prophase stage of cell division, return to interphase and remain so until the influence of colchicine in the cell has subsided.
3. In shoot dip treatment all axillary buds from untreated portions of shoot should be removed.

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VITA

SARUP SINGH SEKHON

Candidate for the Degree of

Master of Science

Thesis: COLCHICINE TREATMENTS TO INDUCE POLYPLOIDY IN GRAPES

Major Field: Horticulture

Biographical:

Personal data: Born near Jamsher, Punjab, August 22, 1922

Education: Attended and matriculated from Khalsa Collegiate School, Amritsar, Punjab, in 1938; received Bachelor of Science in Agriculture degree in 1942 from the University of Punjab; received Master of Science degree from the Agricultural College, Lyallpur, Pakistan, with major in vegetables in 1945; completed requirements for another Masters from Oklahoma Agricultural and Mechanical College in May 1956.

Professional experience: Entered the service of Punjab Agricultural Department in 1946 and served as Incharge Cold storage, and then Incharge Home Demonstration in Fruit and Vegetable Preservation Project. From 1949 to 1953 remained Incharge of the Government Fruit Experimental Station, Attari, Amritsar.